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European Patent Office  
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⑪ Publication number:

0 345 038  
A2

⑫

## EUROPEAN PATENT APPLICATION

⑬ Application number: 89305478.3

⑮ Int. Cl.4: A 61 K 31/19  
A 61 K 31/22

⑭ Date of filing: 31.05.89

⑯ Priority: 02.06.88 GB 8813012

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⑰ Data of publication of application:

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06.12.89 Bulletin 89/49

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⑲ Designated Contracting States:  
AT BE CH DE ES FR GB GR IT LI LU NL SE

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㉑ Non-beta-oxidizable fatty acid analogues with the effect to reduce the concentration of cholesterol and triglycerides in blood of mammals.

㉒ Compounds of general formula (I) below are used for the manufacture of a medicament for the treatment of hypolipaemic conditions and for reducing the concentration of cholesterol and triglycerides in the blood of mammals:

Alkyl-X-CH<sub>2</sub>COOR (I)

wherein Alkyl represents a saturated or unsaturated hydrocarbon group of from 8-22 carbon atoms, X represents O, S, SO or SO<sub>2</sub> and R is hydrogen or C<sub>1</sub>-C<sub>4</sub> alkyl, for the manufacture of a medicament for the treatment of hypolipaemic conditions and for reducing the concentration of cholesterol and triglycerides in the blood of mammals.

EP 0 345 038 A2

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EP 0 345 038 A2

## Description

### NON- $\beta$ -OXIDIZABLE FATTY ACID ANALOGUES WITH THE EFFECT TO REDUCE THE CONCENTRATION OF CHOLESTEROL AND TRIGLYCERIDES IN BLOOD OF MAMMALS

This invention relates to the use of certain non- $\beta$ -oxidizable fatty acid analogues for the manufacture of a medicament for the treatment of hypolipaemic conditions and for reducing the concentration of cholesterol and triglycerides in the blood of mammals.

Excess of lipids in blood has been shown to accelerate the development of arteriosclerosis and is a risk factor for myocardial infarction. Accordingly, a reduction of the concentration of lipids in the blood by diet or by drugs is used as a preventive measure in people at risk due to high blood levels of cholesterol and triglycerides.

It is well known that some natural long-chain fatty acids, particularly polyunsaturated fatty acids of marine origin, are effective in lowering plasma triglyceride and possibly cholesterol levels in man. Experimental studies in animals have shown that these fatty acids enhance fatty acid oxidation, partly by increased peroxisomal activity, depress synthesis of fatty acids, and decrease synthesis of apolipoprotein B and product of VLDL.

Similar effects are obtained with a series of peroxisome proliferating, hypolipaemic drugs like clofibrate, bezafibrate, tiadenol and others which have more complicated chemical structures. These compounds, however, are generally more toxic and have several undesirable side effects.

Considering that the polyunsaturated long chain fatty acids are metabolized relatively slowly, we postulated that simple non- $\beta$ -oxidizable fatty acids analogues might have similar effects. In feeding experiments with such fatty acid analogues the results show that they lower the blood concentration of cholesterol and triglyceride, without any overt toxic effect.

The fatty acid analogues induce some increased peroxisomal  $\beta$ -oxidation activity. These fatty acid analogues are to our best knowledge the simplest lipid-lowering compounds found so far.

Thus, the present invention provides fatty acid analogues with the ability to lower the concentration of cholesterol and triglyceride in the blood and with improved effect relative to the conventional drugs clofibrate, bezafibrate and tiadenol and without the undesirable side effects of these drugs.

More particularly, the present invention is the use of non- $\beta$ -oxidizable fatty acid derivatives represented by the general formula (I):

Alkyl-X-CH<sub>2</sub>COOR (I)

wherein Alkyl represents a saturated or unsaturated hydrocarbon group of from 8-22 carbons atoms, X represents an oxygen atom, a sulfur atom, a sulfoxide (SO) group or a sulfone (SO<sub>2</sub>) group, and R is hydrogen, or C<sub>1</sub>-C<sub>4</sub> alkyl group such as ethyl, for the manufacture of a medicament for the treatment of hypolipaemic conditions and for reducing the concentration of cholesterol and triglycerides in the blood of mammals.

In the preferred embodiments of the present invention "Alkyl" represents the tetradecyl group.

The compounds used according to the present invention wherein X is an oxygen or a sulphur atom may be prepared according to the following general procedure:

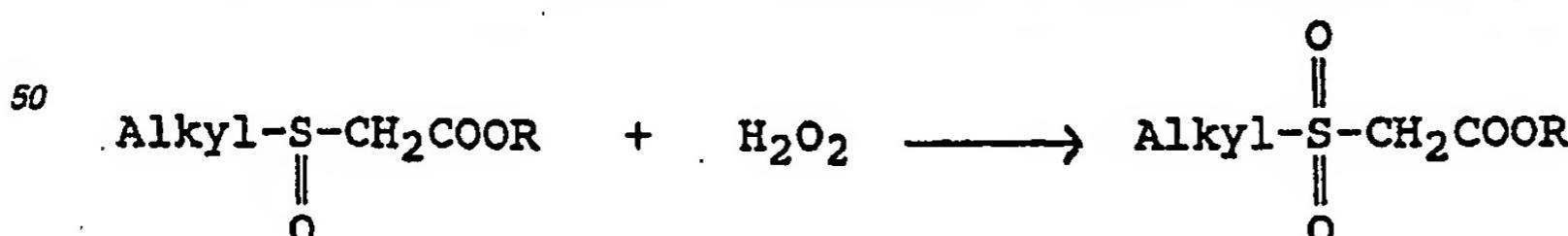


The thio compounds used according to the present invention may be prepared by the general procedure indicated below:

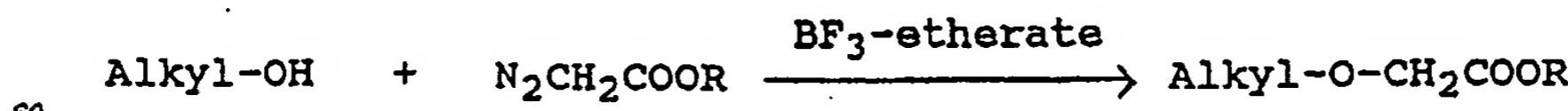


The sulfoxide group-containing compounds may be prepared by oxidation of the corresponding thio compound with an oxidizing agent such as hydrogen peroxide, as represented by the reaction scheme below:  
$$\text{Alkyl-S-CH}_2\text{COOR} + \text{H}_2\text{O}_2 \rightarrow \text{Alkyl-SO-CH}_2\text{COOR}$$

The sulfone group-containing compounds may be prepared by oxidation of the corresponding sulfoxide compound with an oxidizing agent such as hydrogen peroxide, as represented below:



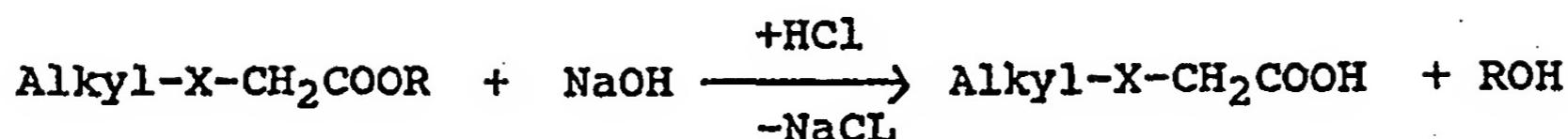
Further, the oxygen ethers used according to the present invention may be prepared according to the procedure shown below:



The conversion of the esters (R being C<sub>1</sub>-C<sub>4</sub> alkyl group) to the corresponding acids may be accomplished using the following well known procedure:

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EP 0 345 038 A2



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The preparation of a number of non- $\beta$ -oxidizable fatty acid derivatives of formula (I) above will now be given by way of illustration.

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## Example 1

### S-Tetradecylthioacetic acid ( $\text{CH}_3(\text{CH}_2)_{13}-\text{S-CH}_2\text{COOH}$ )

KOH, 20 g (0.3 equivalents), mercaptoacetic acid (12 ml, 0.14 equivalents), and tetradecylbromide (25 ml, 0.09 equivalents) were added in that order to 200 ml methanol and stirred overnight under nitrogen. A white precipitate of potassium bromide was formed. To the reaction mixture was added concentrated HCl (30 ml) and water (400 ml). The precipitate sedimented by centrifugation was washed twice with water (750 ml) and dissolved in 500 ml 90% hot methanol. After cooling and crystallisation at room temperature the precipitate was recrystallized from 600 ml hot 75% ethanol. The tetradecylthioacetic acid crystallized as white flakes and was isolated by filtration.

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Yield: 23 g = 75% based on the amount of tetradecylbromide used.

Thin layer chromatography on silica plates with hexane-ethylether formic acid (60:40:1) gave only one spot with iodine vapour,  $R_f$  = approximately 0.6.

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## Example 2

### Tetradecylthioacetic acid sulfoxide ( $\text{CH}_3(\text{CH}_2)_{13}-\overset{\text{O}}{\underset{\text{S}}{\text{S}}}-\text{CH}_2\text{COOH}$ )

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Tetradecylthioacetic acid (5.75 g, 20 mmol) was dissolved in 60 ml acetone. Hydrogen peroxide (2.25 ml, 30%) was added and the mixture left overnight at room temperature. Additional hydrogen peroxide (0.5 ml, 30%) was added and the mixture left for another 24 h. The reaction mixture was evaporated to dryness in vacuo at room temperature. Thin layer chromatography showed some remaining unchanged tetradecylthioacetic acid. Crystallization twice from hot acetone removed the tetradecylthioacetic acid.

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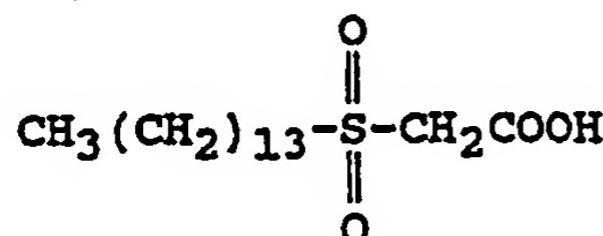
Yield: 4.6 g = 75%.

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## Example 3

### Tetradecylthioacetic acid sulfone

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5.75 tetradecylthioacetic acid was dissolved in 60 ml acetone and hydrogen peroxide (30%, 5 ml) was added. The mixture was left overnight at approximately 40°. To the reaction mixture was added additional 40 ml acetone and 10 ml H<sub>2</sub>O. Upon cooling the sulfone precipitated. Thin layer chromatography on silica plates showed only one spot in iodine vapour (hexane-ethylether-formic acid) (60:40:1).

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$R_f$  values:

tetradecylthioacetic acid	0.6
sulfoxide	0.1
sulfone	0.2

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EP 0 345 038 A2

## Example 4

- 5      Ethyl tetradecyloxy acetate ( $\text{CH}_3(\text{CH}_2)_{13}-\text{O}-\text{COOCH}_2\text{CH}_3$ )  
Tetradecanol (23.6 g, 0.11 Mol) and ethyl diazoacetate (14.4 g, 0.13 Mol) was dissolved in 140 ml dichloromethane and the mixture was cooled to 0°C. Boron trifluoride ethyl etherate (3 ml) dissolved in 10 ml dichloromethane was added to the stirred solution during 10 minutes. Subsequently the solution was stirred at room temperature for 45 minutes and thereafter washed with water and dried ( $\text{MgSO}_4$ ). Dichloromethane was vaporized on a rotavapor and the product was purified by distillation at reduced pressure. Yield 20.5 g (62%), b.p. 120-125°C/0.01 mmHg.
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## Example 5

- 15      Tetradecyloxy acetic acid ( $\text{CH}_3(\text{CH}_2)_{13}\text{OCH}_2\text{COOH}$ )  
Ethyl tetradecyloxyacetate (18.0 g, 0.08 Mol) was dissolved in methanol (80 ml) and a solution of NaOH (8.0 g, 0.20 Mol) dissolved in water (50 ml) was added and the mixture was refluxed for 5 hours. After cooling the reaction mixture was washed with hexan, acidified with hydrochloric acid and extracted with ether. The ether extracts were washed with water and dried over  $\text{MgSO}_4$ . The residue after vaporisation of the solvent was recrystallized from ether/hexan. Yield 14.9 g (91%) m.p. 58-59°C.
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## Experiments

- 25      Male Wistar rats, weighing 180-200 g at the start of the experiment, were housed individually in metal wire cages in a room maintained at 12 h light-dark cycles and a constant temperature of  $20 \pm 3^\circ\text{C}$ . The animals were acclimatized for at least 5 days under these conditions before the start of the experiments.  
Compound I (tetradecylthioacetic acid), and other fatty acid derivatives according to the invention, were suspended in 0.5% (w/v) carboxymethyl cellulose (CMC) and the suspension was micronized by ultrasonication. The drugs were administered by gastric intubation (gavage) once daily for 5 days. Results obtained with compound I are reported in Figure 1 and Figure 2, wherein:  
Fig. 1 shows the effect of the dose of compound I on serum cholesterol (○) and triglycerides (●). \*p 0.05 compared to the control group; and  
Fig. 2 shows the effect of compound I at a dose of 150 mg/day/kg body weight on serum cholesterol (○) and triglycerides (●). \*p 0.05 compared to the control group.  
The hypolipidemic effect as a function of dose administered is shown in Figure 1. A time-course study from 12 h up to 14 days of the hypolipidemic effects were examined with a dose of 150 mg/day/kg body weight (see Figure 2). Four animals were used for each treatment and a 0.5% CMC solution only was administered to rats as control. After administration of the test compound, rats were fasted for 12 hours and anesthetized with haloethan. Blood samples were collected by cardiac puncture, and lipid concentrations in serum were determined using an autoanalyzer. Liver was removed at the same time, and it was homogenized in ice-cold sucrose medium (0.25 M sucrose, 10 mM Hepes buffer, pH 7.4 and mM EDTA). A 10% liver homogenate was made and peroxisomal  $\beta$ -oxidation was measured. The effect of the compounds were compared with that of clofibrate, tiadenol and niadenate. These drugs were suspended and administered at a dose of 150 mg/day/kg body weight to rats in a similar way as compound I. The dosing lasted for 10 days and the data obtained are shown in Table 1.
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Table 1

Effect of compound I and different hypolipidemic drugs on the liver weight and peroxisomal beta-oxidation in rat liver homogenates

Compounds	Liver weight/body weight	Peroxisomal $\beta$ -oxidation (nmol/min/g liver)
Control	4.1 $\pm$ 0.1	340 $\pm$ 30
Compound I	4.3 $\pm$ 0.2	460 $\pm$ 40**
Clofibrate	5.7 $\pm$ 0.2*	1120 $\pm$ 60*
Tiadenol	6.9 $\pm$ 0.3*	1450 $\pm$ 80*
Niadenate	7.3 $\pm$ 0.2*	1480 $\pm$ 90*

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EP 0 345 038 A2

Values represent means  $\pm$  S.D. of four rats. Statistically significant differences from the controls:

- $P < 0.01$ ,
- $P < 0.05$ .

Table 1 clearly shows the superior property of the compound I regarding adverse side effects at doses that give comparable lipid lowering.

Further, Figs. 1 and 2 show that compound I can achieve a significant reduction in serum lipids.

The compounds of general formula (I) thus exhibit a good hypolipidemic effect in blood of mammals such as rats and possess low toxicity measured as increase in liver weight and increased peroxisomal  $\beta$ -oxidation. The compounds exhibit a good hypolipidemic effect in rats, but lower toxicity compared to the comparative drugs (clofibrate, tiadenol and niadenate). Therefore they are potentially useful as medicinal compounds. For such purposes, the compounds of the present invention can be administered orally or parenterally in a conventional dosage form such as tablets, capsules, powders, emulsions and solutions prepared according to conventional pharmaceutical practices.

The compounds used according to the present invention may be administered to patients suffering from any type of dyslipidaemia except type I.

The dosage range for the compounds according to the present application is contemplated to be from 100 to 1000 mg/day for the average adult patient. Of course, the actual dose necessary will depend on the patient's condition and will have to be determined by the attending physician from case-to-case.

The compounds of general formula (I) may be formulated in any suitable pharmaceutical composition, alone or in admixture with the commonly used pharmaceutical carrier materials, in the preparation of a medicament for administration. For oral pharmaceutical compositions such carrier materials as, for example, water, gelatin, gums, lactose, starches, magnesium stearate, talc, oils, polyalkylene glycol, petroleum jelly and the like may be used. Such pharmaceutical preparations may be in unit dosage form and may additionally contain other therapeutically valuable substances or conventional pharmaceutical adjuvants such as preservatives, stabilizing agents, wetting agents, emulsifying agents, buffers and the like. The pharmaceutical preparations may be in conventional solid dosage forms such as tablets, capsules, dragees and the like, in conventional liquid forms such as solutions, suspensions, emulsions and the like, and other conventional dosage forms such as dry ampules, suppositories and the like.

For parenteral administration the compounds according to the present invention may be administered as solutions, suspensions or emulsions using conventional pharmaceutical carrier materials such as for example water for injection, oils, polyalkylene glycols and the like. These pharmaceutical preparations may further include conventional pharmaceutical adjuvants such as preservatives, stabilizing agents, wetting agents, emulsifying agents, salts for the adjustment of the osmotic pressure, buffers and the like. The preparations may also contain other therapeutically active materials.

## Claims

1. Use of non- $\beta$ -oxidizable fatty acid analogues of the general formula (I):

Alkyl-X-CH<sub>2</sub>COOR (I)

wherein Alkyl represents a saturated or unsaturated hydrocarbon group of from 8-22 carbon atoms, X represents O, S, SO or SO<sub>2</sub> and R is hydrogen or C<sub>1</sub>-C<sub>4</sub> alkyl, for the manufacture of a medicament for the treatment of hypolipaemic conditions and for reducing the concentration of cholesterol and triglycerides in the blood of mammals.

2. Use according to Claim 1 wherein Alkyl represents the tetradecyl group.

3. Use according to Claim 2, wherein the compound of formula (I) is S-tetradecylthioacetic acid.

4. Use according to Claim 2, wherein the compound of formula (I) is tetradecylthioacetic acid sulfoxide.

5. Use according to Claim 2, wherein the compound of formula (I) is tetradecylthioacetic acid sulfone.

6. Use according to Claim 2, wherein the compound of formula (I) is ethyl tetradecyloxy acetate.

7. Use according to Claim 2 wherein the compound of formula (I) is tetradecyloxy acetic acid.

8. A process for the manufacture of a medicament for the treatment of hypolipaemic conditions and for reducing the concentration of cholesterol and triglycerides in the blood of mammals, comprising incorporating with a pharmaceutically acceptable carrier or diluent a non- $\beta$ -oxidizable fatty acid analogue of the general formula (I):

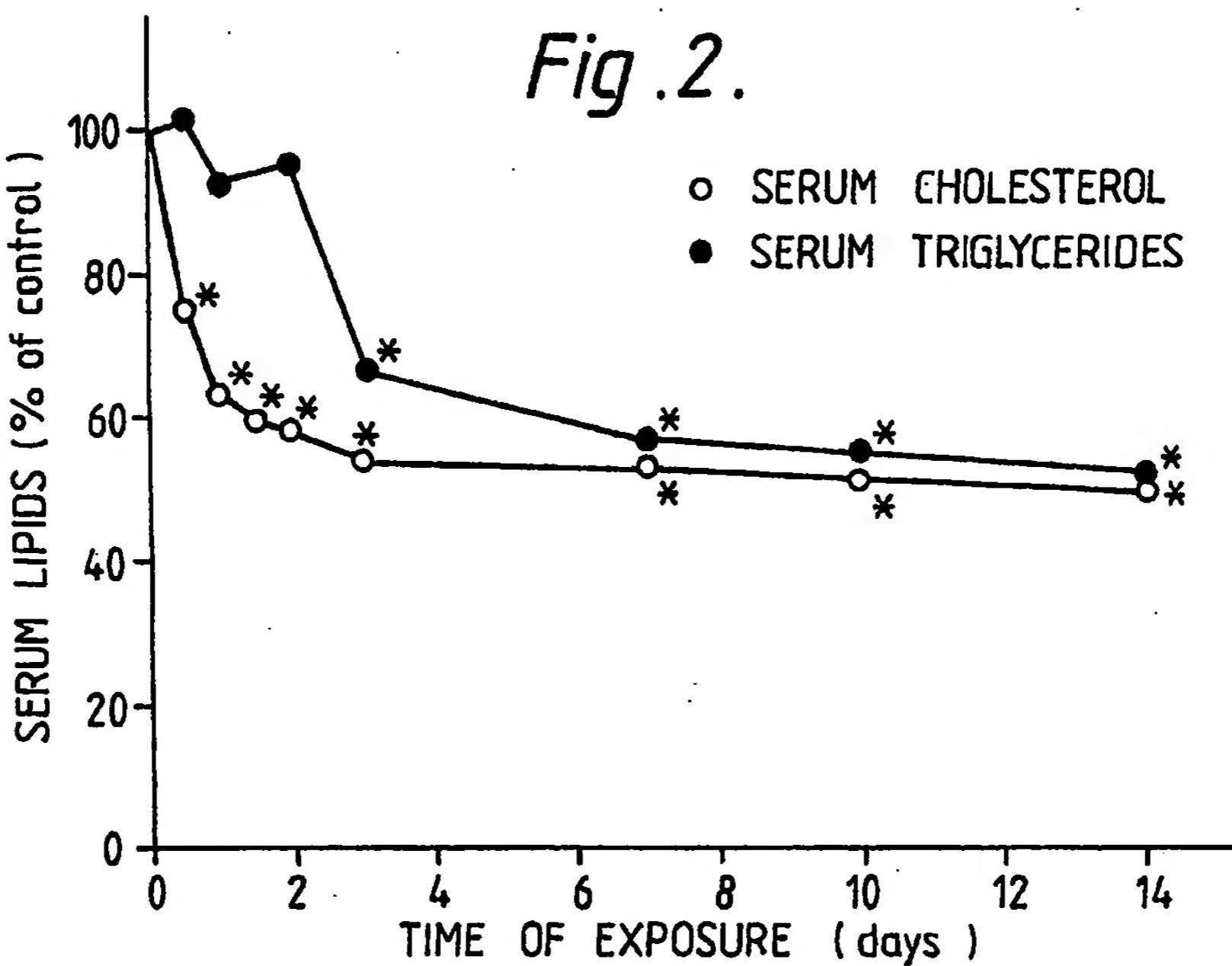
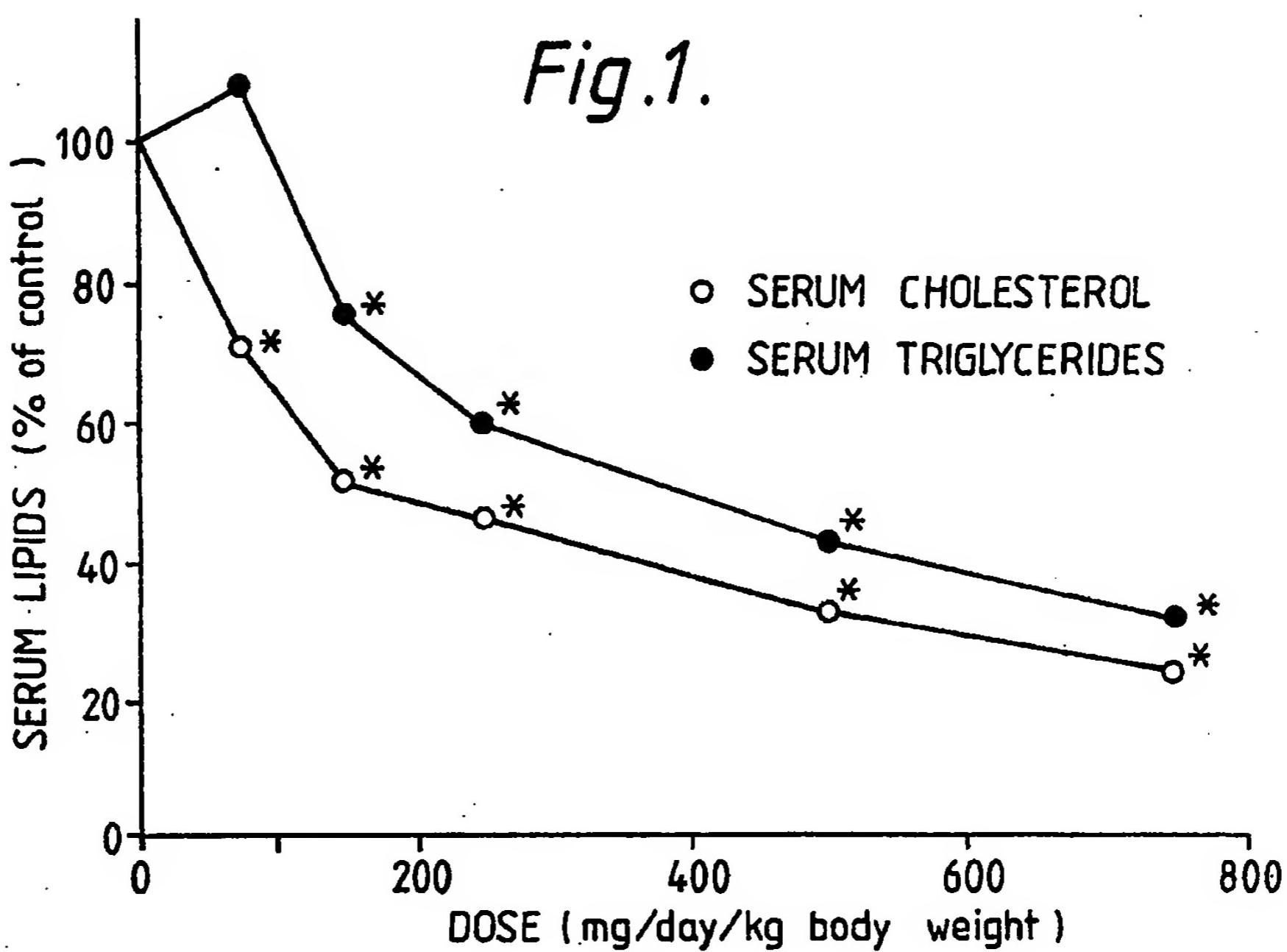
Alkyl-X-CH<sub>2</sub>COOR

wherein Alkyl represents a saturated or unsaturated hydrocarbon group of from 8-22 carbon atoms, X represents O, S, SO or SO<sub>2</sub>, and R is hydrogen or C<sub>1</sub>-C<sub>4</sub> alkyl.

9. A process according to Claim 8, wherein the compound of general formula (I) is as defined in any one of Claims 2-7.

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EP 0 345 038 A2



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(11) Publication number: 0 345 038 A3

(22)

## EUROPEAN PATENT APPLICATION

(21) Application number: 89305478.3

(51) Int. Cl. 5: A61K 31/19, A61K 31/22

(22) Date of filing: 31.05.89

(30) Priority: 02.06.88 GB 8813012

(43) Date of publication of application:  
06.12.89 Bulletin 89/49

(84) Designated Contracting States:  
AT BE CH DE ES FR GB GR IT LI LU NL SE

(88) Date of deferred publication of the search report:  
12.06.91 Bulletin 91/24

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(54) Non-beta-oxidizable fatty acid analogues with the effect to reduce the concentration of cholesterol and triglycerides in blood of mammals.

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EP 0 345 038 A3

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## EUROPEAN SEARCH REPORT

Application Number

EP 89 30 5478

DOCUMENTS CONSIDERED TO BE RELEVANT		Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. CL.5)
Category	Citation of document with indication, where appropriate, of relevant passages		
A	DE-A-2 858 683 (NATTERMANN) * Claims *	1-9	A 61 K 31/19 A 61 K 31/22
A	NL-A-7 405 744 (DOW CHEMICAL CO.) * Page 1, line 1 - page 2, line 15; claims *	1-9	
A	CH-A-6 612 07 (HOERRMANN) * Whole document *	1-9	
A	DE-A-2 515 148 (BAYER) * Pages 1,2,3,4; claims *	1-9	
		TECHNICAL FIELDS SEARCHED (Int. CL.5)	
		A 61 K	
The present search report has been drawn up for all claims			
Place of search	Date of completion of search	Examiner	
The Hague	19 March 91	GOETZ G.	
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